4-(METHYLNITROSAMINO)-1-(3-PYRIDYL)-1-BUTANONE (NNK)[1]

1. Chemical and Physical Data

1.1 Synonyms and trade names

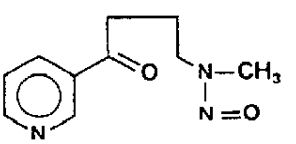
Chem. Abstr. Services Reg. No.: 64091-91-4

Chem. Abstr. Name: 1-Butanone, 4-(methylnitrosoamino)-1-(3-pyridinyl)-

IUPAC Systematic Name: 4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone

Synonym: 4-(*N*-Methyl-*N*-nitrosamino)-1-(3-pyridyl)-1-butanone

1.2 Structural and molecular formulae and molecular weight



$C_{10}H_{13}N_3O_2$ Mol. wt: 207.2

1.3 Chemical and physical properties of the pure substance

(*a*) *Description*: Light-yellow crystalline solid

(*b*) *Melting-point*: 63-65°C (Hecht *et al.*, 1977)

(*c*) *Spectroscopy data*: Infrared, nuclear magnetic resonance (Hecht *et al.*, 1977) and mass spectroscopy data (Hecht *et al.*, 1981) have been reported.

(*d*) *Reactivity*: Reacts with isopropyl nitrite in a saturated solution of hydrogen chloride in methanol to form an α-oxime (Hecht *et al.*, 1978a). Can be reduced with sodium borohydride to 4-(methylnitrosamino)-1-(3-pyridyl)butan-1-ol (NNAl)[2]. Can be oxidized with *meta*-chloroperbenzoic acid to give 4-(methylnitrosamino-1-(3-pyridyl-*N*-oxide)-1-butanone (NNK-*N*-oxide) (Hecht *et al.*, 1980a)

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[1]The abbreviation NNK was selected to emphasize the relationship of this compound to nicotine, and stands for 'nicotine-derived nitrosamino ketone'.

[2]NNAl, nicotine-derived nitrosamino alcohol

1.4 Technical products and impurities

This compound is not produced commercially.

Synthetic NNK and NNK isolated from tobacco are mixtures of 72.7% E-isomer and 27.3% Z-isomer (Hecht *et al.*, 1977; Hoffmann *et al.*, 1980a).

2. Production, Use, Occurrence and Analysis

2.1 Production and use

NNK was prepared by Hecht *et al.* (1977) by the reaction of sodium hydroxide and sodium nitrite with 4-(*N*-methyl)-1-(3-pyridyl)-1-butanone dihydrochloride.

NNK was obtained by Hecht *et al.* (1978b) as one of several reaction products when nicotine was allowed to react with 1.4 equivalents of sodium nitrite in aqueous solution at 20°C for 17 h. The highest yield of NNK at pH 4.5 was only 0.5% of the theoretical amount. When nicotine was reacted with five equivalents of sodium nitrite at 90°C for 3 h at pH 3.4-4.2, a 2.3% yield of NNK was obtained.

No evidence was found that NNK has ever been produced in commercial quantities or that it has any uses other than as a laboratory chemical.

2.2 Occurrence

(a) Tobacco and tobacco smoke

NNK is formed by oxidation and nitrosation of nicotine, during the curing, ageing, processing and smoking of tobacco. NNK has been found in tobacco at levels of 0.1-35 mg/kg, in snuff products at 0.2-8.3 mg/kg, and in cigarette smoke at 0.1-0.5 µg/cigarette. Additional quantities of NNK may be formed in the oral cavity during oral use of snuff or tobacco chewing (Hoffmann *et al.*, 1979, 1980b; Hoffmann & Adams, 1981; Hoffmann *et al.*, 1982a; Brunnemann *et al.*, 1983; Hoffmann & Hecht, 1983).

Table 1 summarizes the data obtained from analyses of the smoke of selected commercial cigarettes with and without filter tips and shows the effectiveness of the filters of cigarettes D and E in decreasing the occurrence levels of NNK (Hoffmann *et al.*, 1982b).

Nicotine and NNK concentrations found in cigarette and cigar tobaccos, in both their mainstream (during puff drawing) and sidestream (generated during smouldering of tobacco in between puffs) smoke and in chewing tobacco are presented in Table 2.

Studies using tracer compounds [carbonyl-^{14}C]NNK (Castonguay *et al.*, 1984a) demonstrated that 6.9-11.0% of the NNK formed in tobacco during the curing process transfer into the cigarette mainstream smoke. This constitutes 26-37% of the NNK present in the mainstream smoke. Thus, 63-74% of NNK in the cigarette mainstream smoke are formed during smoking. The cigarette types used in this study were a US commercial non-filter cigarette and three experimental non-filter cigarettes (burley tobacco, flue-cured tobacco and tobacco blend) (Adams *et al.*, 1983b).

Table 1. Reduction of nicotine and NNK concentrations in cigarette smoke by filtration[a]

Cigarette	Length smoked/length of cigarette (mm)	Nicotine (mg/cigarette)[b]	NNK (μg/cigarette)[b]
A NF	50/65	1.82	0.7
F	50/85	1.10 (-40%)	0.4 (-50%)
B NF	50/65	1.84	0.5
F	50/85	1.31 (-29%)	0.3 (-48%)
C NF	50/65	1.66	0.7
F	50/85	1.15 (-30%)	0.4 (-45%)
D NF	50/65	1.60	0.8
F	50/85	1.05 (-34%)	0.2 (-73%)
E[c] NF	50/73	1.63	0.9
F	50/100	1.08 (-34%)	0.3 (-71%)

[a]Data from Hoffmann *et al.* (1982b)
[b]In parentheses, percentage changes in yields on comparing filtered (F) and non-filtered (NF) smoke
[c]Perforated filter tip

Table 2. NNK concentrations in cigarettes, cigars and chewing tobacco, and in cigarette and cigar mainstream and sidestream smoke[a]

Tobacco product[b]	NNK concentration		
	In tobacco (mg/kg)	In mainstream smoke (μg/cig)	In sidestream smoke (μg/cig)
Burley cigarette, NF	ND[c]	0.3	0.7
Bright cigarette, NF	0.4	0.4	0.5
Commercial cigarette, NF	0.7	0.1	0.4
Commercial cigarette, FA	0.7	0.2	0.2
Kentucky 1R1, NF	0.1	0.2	0.2
US cigarette, NF[d]	–	0.8	–
US cigarette, NF[e]	1.4	0.2	–
German (Federal Republic) cigarettes[f]			
Brand A, NF	–	0.08	–
Brand B, NF	–	0.04	–
Brand C, FA	–	0.06	–
Brand D, FA		0.02	–
Brand E, FA	–	0.05	–
Brand F, FA	-	0.06	–
Commercial French cigarette, NF, 70 mm	0.5	0.4	–
Commercial French cigarettes, FA, 70 mm	0.4	0.4	–
NF	1.1	0.4	–
FA	1.1	0.2	–
FP	1.1	0.1	–
Little cigar, FA	35.0	4.2	0.8
Cigar (Colombian tobacco) (5.7 g)	1.1	1.9	15.7
Fine-cut chewing tobacco	2.4	NA[g]	NA
Fine-cut chewing tobacco[d]	7.4	NA	NA

[a]Data from Hoffmann *et al.* (1980b), unless otherwise noted
[b]All cigarettes and the little cigar were 85 mm long, unless otherwise noted. Abbreviations: NF, non-filter; FA, cellulose acetate filter; FP, paper filter
[c]ND, not detected
[d]Data from Adams *et al.* (1983a); cigarettes and tobacco used were purchased in Westchester County, NY, in 1981
[e]Data from Adams *et al.* (1983b); cigarettes used were purchased in Westchester County, NY, in 1981
[f]Data from Rühl *et al.* (1980); cigarettes were popular brands purchased in Berlin in 1979
[g]NA, not applicable

NNK concentrations found after analysis of snuff obtained in Sweden, Denmark, the Federal Republic of Germany and the USA were higher than those in cigarette tobacco presented above. The results are summarized in Table 3. NNK concentrations differed not only among snuff brands but also in samples of the same brand bought in different cities. The latter differences were attributed to possible variations in NNK content between batches and/or effects of ageing. The effects of ageing were demonstrated by opening individual portions (packed in aluminium foil) and storing the snuff in the open air: within eight days, the NNK content had increased by 48% and then remained stable (Hoffmann & Adams, 1981; Hoffmann *et al.*, 1982b).

Table 3. Nicotine and NNK concentrations in commercial snuff[a]

Snuff origin	Type of packaging[b]	Nicotine (%)	NNK (mg/kg)[c]
USA			
Brand I New York and Tennessee	A	2.4	2.4
Brand II New York and Tennessee	A	2.3	4.7
Brand III New York and Tennessee	B	1.5	1.3
Federal Republic of Germany			
Brand I Munich	C	0.6	1.5
Brand II Munich	C	0.5	1.5
Sweden			
Brand I Umeå	A	1.5	2.2
Brand I Uppsala	A	1.5	1.4
Brand I Lund	A	1.5	2.3
Brand II Umeå	A	1.8	1.8
Brand II Uppsala	A	1.8	0.6
Brand II Lund	A	1.8	2.1
Brand III Umeå	A	0.6	2.5
Brand III Uppsala	A	0.6	0.9
Brand III Lund	A	0.7	0.9
Brand IV Umeå	A	1.1	2.1
Brand IV Uppsala	A	1.1	3.8
Brand IV Lund	A	1.1	1.6
Brand V Umeå	D	2.2	1.3
Denmark			
Brand I Copenhagen	E	1.1	2.0
Brand II Copenhagen	E	2.1	1.4
Brand III Copenhagen	E	3.1	7.0
Ageing test[d]: 0 time	-	-	1.3
8 days	-	-	1.9

[a]Data from Hoffmann and Adams (1981) and Hoffmann *et al.* (1982b); values are given for dry snuff, moisture content about 50%

[b]Abbreviations: A, waxed-paper container with metallic lid, containing approximately 50 g; B, 25 individual portions of approximately 11 g packaged in paper in a plastic container; C, plastic foil-lined aluminium bags containing 100 g; D, individual snuff portion in a paper bag packaged in a crimped airtight aluminium envelope, with 10 envelopes in a plastic bag, amounting to approximately 10 g; E, hard-plastic container

[c]NNK values are averages of three runs.

[d]The aluminium foil-wrapped package (Swedish brand V) was opened at '0 time'.

(b) *Human tissues and secretions*

Saliva was examined from women who had been long-term oral-snuff users and who were employed in two southern US furniture companies. The wide range of NNK concentrations (2.1-201 ng/g) in the saliva of individual users indicated that during oral use of snuff NNK is extracted from the tobacco plug at varying rates (Hoffmann & Adams, 1981).

In another study, saliva of four women who were long-term (>10 years) oral-snuff users was analysed on two different days after they had used a specific brand of snuff with known levels of NNK. NNK and nicotine levels in saliva (Table 4) varied significantly between subjects, as well as between samples from the same individual taken on different days. The variations in NNK values were at least partially explained by differences in the intensity with which individuals practised the habit at different times, and perhaps are also due to varying rates of salivation (Hoffmann & Adams, 1981; Hoffmann *et al.* 1982b).

Table 4. Nicotine and NNK concentrations in snuff and in saliva of women who were long-term oral-snuff users[a]

Subject	Age (years)	Snuff		Saliva[b]		
		Nicotine (mg/g)	NNK (μg/g)	Day of sampling	Nicotine (mg/g)	NNK (μg/g)
1	41	23.4	4.7	1	0.2	0.026
				2	0.5	0.021
2	37	23.4	4.7	1	0.07	0.013
				2	0.4	<0.010
3	44	23.4	4.3	1	1.2	0.096
				2	1.6	0.062
4	52	23.6	5.2	1	0.2	0.01
				2	0.4	0.023

[a]Data from Hoffmann and Adams (1981) and Hoffmann *et al.* (1982b)

[b]Saliva of three women who did not use snuff (controls) was free of nicotine and tobacco-specific *N*-nitrosamines.

NNK was found in the saliva of chewers of betel quid with tobacco at levels of 1.0-2.3 ng/g [mean, 1.5 ng/g] (Wenke *et al.*, 1984) and up to 2.3 ng/ml (mean, 0.34 ng/ml) (Nair *et al.*, 1985).

2.3 Analysis

Standard methods for the analysis of NNK are described in detail in an IARC manual on selected methods of analysis (Egan *et al.*, 1983).

3. Biological Data Relevant to the Evaluation of Carcinogenic Risk to Humans

3.1 Carcinogenicity studies in animals

(a) *Subcutaneous and/or intramuscular administration*

Rat: Groups of 12 male and 12 female Fischer 344 rats, seven weeks of age, received thrice-weekly s.c. injections of 11.7 mg [0.06 mmol] NNK (purity, >99%) in 0.3 ml trioctanoin for 20 weeks (total dose, 702 mg; 3.5 mmol). Animals were maintained until they died spontaneously or were killed after 12 months. Malignant tumours of the nasal cavity (olfactory neuroblastomas and rhabdomyosarcomas) were observed in 6/12 males and in 4/12 females; malignant tumours of the lung (an adenocarcinoma and adenosquamous-cell carcin-

omas) in 5/12 males; and malignant liver tumours (hepatocellular carcinomas and haemangiosarcomas) in 7/12 males and 12/12 females. No tumour of the nasal cavity, lung or liver was found in 12 vehicle controls that received trioctanoin only (Hecht *et al.*, 1980b).

Groups of 15-27 male and 15-27 female Fischer 344 rats, nine weeks of age, received thrice weekly s.c. injections of NNK (purity, >99%; Hecht *et al.*, 1977) in trioctanoin (total doses, approximately 9.0, 3.0 and 1.0 mmol/kg bw), or trioctanoin alone (vehicle controls; 27 males and 27 females) for 20 weeks (with a two-week interruption after seven weeks of injections). Animals were killed when moribund or when only 20% of rats in a group were still alive (the experiment was ended at 70-120 weeks). No difference in body weight was seen among treated and control animals. By 80 weeks, all animals in the high-dose group had died; all animals in the other two groups had died by 120 and 130 weeks, respectively. The incidences of malignant tumours in the nasal cavity (aesthesioneuroepitheliomas, squamous-cell carcinomas, anaplastic carcinomas, spindle-cell sarcomas) and of benign tumours (squamous- and transitional-cell papillomas and polyps) in males and females of the high-, medium- and low-dose groups were 14/15 and 14/15, 13/15 and 12/15, and 20/27 and 10/27, respectively. Malignant tumours predominated in the high-dose group (21/28), but nasal-cavity tumours in the low-dose group were mostly benign (29/30). Incidences of lung tumours (adenomas, adenocarcinomas, squamous-cell carcinomas) for males and females in the high-, medium- and low-dose groups were 14/15 and 9/15, 13/15 and 7/15, and 23/27 and 8/27, respectively. These incidences all differed significantly from those in vehicle-control rats who received trioctanoin only: none had a nasal-cavity tumour and 1/52 had a lung adenoma. The incidences of benign and malignant liver tumours in treated males and females in the high-, medium- and low-dose groups were 6/15 and 5/15, 4/15 and 4/15, and 3/27 and 4/27, respectively. Of the vehicle-control rats, 3/26 males and 1/26 females had benign liver tumours. Among the 114 NNK-treated rats, only two benign oesophageal tumours were observed; none were observed in controls (Hoffmann *et al.*, 1984). [The Working Group interpreted these results as showing dose-response relationships for induction of tumours of the nasal cavity, lung and liver.]

Hamster: Groups of 15 male and 15 female Syrian golden hamsters, aged eight to 10 weeks, received thrice-weekly s.c. injections of 10 mg (0.048 mmol) NNK (purity, >99%) in 0.3 ml trioctanoin for 6.3 weeks (19 injections; total dose, 190 mg [0.91 mmol]); a second group of 10 males and 10 females, aged eight to 10 weeks, received thrice-weekly s.c. injections of 2.5 mg (0.012 mmol) NNK in 0.3 ml trioctanoin for 25 weeks (75 injections; total dose, 190 mg [0.91 mmol]). Hamsters were maintained until they died or were killed after 16 months (first group and vehicle controls) or 17 months (second group). There was extensive early mortality in the first group, with only 4/15 male and 4/15 female hamsters still alive after 14 weeks; in contrast, the percentages surviving in the second group after seven, 10 and 13 months were 80%, 75% and 30%, respectively. Among 15 male and 15 female vehicle controls, the only tumours observed were a sarcoma at the site of injection and an adrenocortical adenoma. In the first treated group, 22/30 hamsters had respiratory-tract tumours: 10 males with one adenocarcinoma and seven adenomas of the lung and two pleomorphic carcinomas of the nasal cavity; and 12 females with three adenocarcinomas and eight adenomas of the lung and one pleomorphic carcinoma of the nasal cavity. Of these, 14 hamsters with lung adenomas and one with lung adenocarcinoma died within the first 11 weeks of the experiment. In the second group, NNK induced tumours of the lung, trachea or nasal cavity in 18/20 animals (males and females). These included four adenomas and six adenocarcinomas of the lung, five pleomorphic carcinomas and one papilloma of the nasal cavity and three tumours of the trachea in males; and four adenocarcinomas and two adenomas of the lung, five pleomorphic carcinomas of the nasal cavity and four tumours of the trachea in females. In both groups, there were a number of malignant tumours at other sites, and several hamsters had multiple tumours (Hoffmann *et al.*, 1981).

Groups of 10 male and 9-10 female Syrian golden hamsters, eight weeks of age, received single s.c. injections of 1.0 mg (4.8 μmol), 3.3 mg (15.9 μmol) or 10 mg (48.3 μmol) NNK (purity, >99%) in 0.3 ml trioctanoin and were exposed one week later to cigarette smoke or underwent sham exposure twice daily for 69 weeks, at which time surviving hamsters were killed. No tumour of the lung, trachea or nasal mucosa was observed in vehicle-control hamsters exposed to smoke or sham smoking. In contrast, hamsters in all groups treated with NNK had tumours of the respiratory tract (lung, nasal cavity or trachea); and in six (five groups exposed to cigarette smoke and one group sham exposed) of the 12 NNK-treated groups the numbers of animals with tumours significantly exceeded that among controls. Of the hamsters not exposed to smoke, 4/10 males given 10 mg NNK had tumours, including three with lung adenomas and two with papillomas of the nasal mucosa, and 1/9 females (a mucoepidermoid carcinoma of the lung and an olfactory neuroblastoma). Of the hamsters given 3.3 mg NNK, 2/10 males had tumours of the lung (1, an adenocarcinoma) and 1/10 females had a nasal papilloma. Of those given 1 mg NNK, 3/10 males had tumours, including two lung adenomas, one nasal-mucosa papilloma and one tumour of the trachea, and 3/10 females, including two lung adenomas and one nasal-mucosa papilloma (Hecht *et al.*, 1983a).

(*b*) *Intraperitoneal administration*

Mouse: In a screening study for potential carcinogenicity using pulmonary adenomas as an end-point in strain A mice, a group of 25 female strain A/J mice, six to eight weeks old, received thrice-weekly i.p. injections of 0.1 ml of a 1.0% solution of NNK (purity, >99%; Hecht *et al.*, 1977) in trioctanoin over 7.3 weeks (22 injections; total dose, 22 mg; 0.11 mmol) and were killed 30 weeks later. Lung adenomas were observed in 1/25 untreated controls, 5/24 vehicle controls, and 20/23 NNK-treated mice (2.6 lung tumours per animal). No lung adenocarcinoma or malignant tumour at any other site was seen (Hecht *et al.*, 1978c).

A group of 25 female strain A/J mice, six to eight weeks old, received thrice-weekly i.p. injections of 0.1 ml of a solution of NNK (purity, >99%) in 0.9% trioctanoin for 7.3 weeks (22 injections; total dose, 23 mg; 0.11 mmol) and were killed 30 weeks later. All of the 23 surviving, NNK-treated mice had lung tumours, with an average of 37.6 ± 11.8 per mouse. Of the total of 865 lung tumours, 412 were classified as carcinomas. In addition, one mouse had three liver adenomas and two hepatocellular carcinomas and one had a squamous-cell papilloma of the nasal cavity. In comparison, of 25 vehicle controls, four had lung tumours, with a total of five adenomas and no carcinoma. No other tumour was found in this group. In a group of 25 untreated controls, 10 had lung tumours including 16 adenomas and two carcinomas (Castonguay *et al.*, 1983a).

(*c*) *Carcinogenicity of metabolites*

Mouse: In a screening assay for potential carcinogenicity using pulmonary adenomas as an endpoint in strain A mice, a group of 25 female strain A/J mice, six to eight weeks old, received thrice-weekly i.p. injections of NNK-1-*N*-oxide (purity, >99%; Hecht *et al.*, 1980a) or 4-(methylnitrosamino)-1-(3-pyridyl)butan-1-ol (NNAl)[1] (purity, 99.8%) in 0.1 ml trioctanoin for 7.3 weeks (22 injections; total dose, 23 mg; 0.11 mmol) and were killed 30 weeks later. Lung adenomas were observed in 25/25 NNAl-treated mice (with an average of 26.3 ± 11.7 tumours per mouse), 24/25 NNK-1-*N*-oxide-treated mice (3.6 ± 2.7 tumours/mouse), 4/25 trioctanoin-treated controls (0.2 ± 0.5 tumours/mouse) and 10/25 untreated controls (0.6 ± 0.9 tumours per mouse) (Castonguay *et al.*, 1983a).

[1]NNAl, nicotine-derived nitrosamino alcohol; see also Fig. 1, p. 217.

3.2 Other relevant biological data

(a) *Experimental systems*

Toxic effects

No data were available to the Working Group.

Effects on reproduction and prenatal toxicity

No data were available to the Working Group on effects on reproduction.

Pregnant C57BL mice were given intravenous injections of 7.0 mg/kg bw [carbonyl-^{14}C]NNK (Castonguay & Hecht, 1985) on days 13, 16 or 18 of gestation and killed at intervals from 5 min to 8 h. Whole-body autoradiograms revealed the presence of radioactivity in various tissues of the mother, in foetal kidneys and urinary bladder, and in the amniotic fluid. NNK and its carbonyl-reduced metabolite, 4-(methylnitrosamino)-1-(3-pyridyl)butan-1-ol (NNAl)[1], were present in the placentas and foetal tissues; some NNK metabolites were covalently bound to tissues of the nose, lung and liver of 18-day-old foetuses. This was shown *in vitro* to be due to the capacity of these foetal tissues to activate NNK metabolically. Enzymatic α-hydroxylation of NNK did not occur in 13-day-old foetal tissue but did so in 16- and 18-day-old foetal tissue. These results demonstrate that NNK and NNAl can cross the placental barrier and be activated by foetal tissues (Castonguay *et al.*, 1984a).

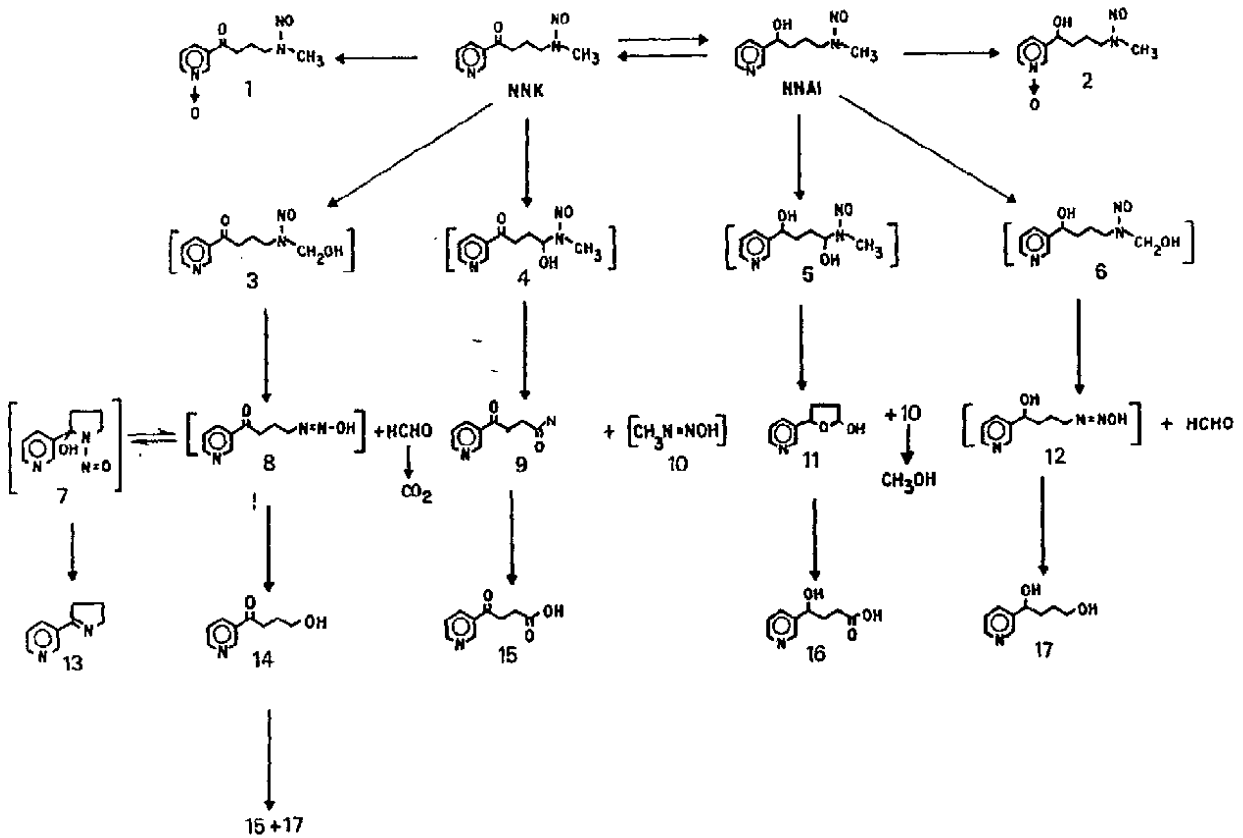
Absorption, distribution, excretion and metabolism

Male Fischer 344 rats were administered 3.5 mg/kg bw [carbonyl-^{14}C]NNK intravenously and killed at various intervals for whole-body autoradiographic studies. After one minute, radioactivity was distributed in most tissues at levels similar to that in blood. Radioactivity exceeding background levels was observed in the mucosa of the ethmoturbinates, the lateral nasal gland, liver, bronchial mucosa, adrenal cortex, preputial gland, submaxillary salivary gland and contents of the stomach. Four and 24 h after injection, these tissues still showed relatively high labelling. Non-extractable, tissue-bound radioactivity was present in the nasal tissues, bronchi and liver. After oral administration of 3.5 mg/kg bw [carbonyl-^{14}C]NNK, the distribution of radioactivity was similar to that observed after intravenous administration, except that there was more labelling in the mouth, oesophagus and upper gastrointestinal tract (Castonguay *et al.*, 1983b). Syrian golden hamsters were given intravenous or subcutaneous injections of 4.9 mg/kg bw [carbonyl-^{14}C]NNK and killed at various intervals for whole-body autoradiography; bound radioactivity was present in the tracheo-bronchial mucosa, nasal mucosa, liver and lateral nasal glands (Tjälve & Castonguay, 1983).

Male Fischer 344 rats given 7.1 μg/kg bw [carbonyl-^{14}C]NNK orally excreted 88% of the dose in 48-h urine, 3% in faeces and <0.5% in expired air; after oral administration of 5.2 μg/kg bw [$^{14}CH_3$]NNK, 39% of the dose was excreted in 48-h urine, 8% in faeces, and 47% in expired air as $^{14}CO_2$ (Castonguay *et al.*, 1983b). Male Syrian golden hamsters given 10 mg (59 mg/kg bw) [carbonyl-^{14}C]NNK subcutaneously excreted 96-98% of the radioactivity in 48-h urine and the remainder in faeces (Hoffmann *et al.*, 1981).

[1]NNAl, nicotine-derived nitrosamino alcohol; see also Fig. 1, p. 217.

Fig. 1. Metabolic transformations of NNK. Structures in brackets represent hypothetical intermediates[a]



[a]Adapted from Castonguay *et al.* (1983a) and Hecht *et al.* (1983b); Compounds: *1*, 1-(methylnitrosamino)-1-(3-pyridyl-*N*-oxide)-1-butanone; *2*, 4-(methylnitrosamino)-1-(3-pyridyl-*N*-oxide)butan-1-ol; *9*, 4-oxo-4-(3-pyridyl)butanal; *11*, 2-hydroxy-5-(3-pyridyl)tetrahydrofuran; *13*, myosmine; *14*, 4-hydroxy-1-(3-pyridyl)-1-butanone; *15*, 4-oxo-4-(3-pyridyl)butyric acid; *16*, 4-hydroxy-4-(3-pyridyl)butyric acid; *17*, 4-hydroxy-4-(3-pyridyl)-1-butanol

Metabolic pathways of NNK are summarized in Figure 1. Three types of metabolic reaction have been established; reduction of the carbonyl group to give 4-(methylnitrosamino)-1-(3-pyridyl)butan-1-ol (NNAl), hydroxylation of the methyl and methylene carbons adjacent (α-) to the *N*-nitroso group, to yield the unstable α-hydroxy-*N*-nitrosamines *3* and *4*, and oxidation of the pyridine nitrogen, yielding compound *1*. NNAl also undergoes α-hydroxylation, leading initially to compounds *5* and *6*, and pyridine-*N*-oxidation, giving compound *2*. Each of these pathways is discussed below in further detail.

NNAl, a major hepatic microsomal metabolite, is found in the urine of NNK-treated Fischer 344 rats (10% of the dose) and Syrian golden hamsters (7% of the dose). It is formed rapidly after administration of NNK to rats or hamsters in blood serum, stomach contents and most tissues at levels that exceed those of NNK. It is also formed rapidly in cultured A/J mouse peripheral lung treated with NNK. NNAl can be reoxidized to NNK *in vitro* and *in vivo* (Hecht *et al.*, 1980a; Hoffmann *et al.*, 1981; Castonguay *et al.*, 1983a,b; Adams *et al.*, 1984).

α-Hydroxylation is thought to be the major activation pathway of NNK. The unstable α-hydroxy-*N*-nitrosamine *4* decomposes to the keto-aldehyde *9*, a rat hepatic microsomal metabolite of NNK, and a methylating agent, presumed to be methyldiazohydroxide (*10*). The mutagenic methylating agent *10* is structurally similar or identical to the methylating agents formed from such well-known carcinogens as *N*-nitrosodimethylamine and *N*-methyl-*N*'-nitrosourea. Thus, administration of NNK to experimental animals should result in formation of O^6-methylguanine and 7-methylguanine in DNA (Hecht *et al.*, 1980, 1983b). This has been confirmed in Fischer 344 rats treated intravenously with 85 mg/kg bw NNK; O^6-methylguanine and 7-methylguanine were detected in the DNA of liver and lung — organs susceptible to the carcinogenic effects of NNK (Castonguay *et al.*, 1984b). Nasal mucosa of Fischer 344 rats cultured with NNK was shown to have a high capacity to hydroxylate the α-carbon of NNK (Brittebo *et al.*, 1983).

The unstable α-hydroxy-*N*-nitrosamine *3* spontaneously decomposes to formaldehyde and the electrophilic diazohydroxide *8*, which induces mutations in *Salmonella typhimurium* (Hecht *et al.*, 1983b). The diazohydroxide *8* reacts with water, yielding keto-alcohol *14*, a hepatic microsomal metabolite of NNK (Hecht *et al.*, 1980a). In Fischer 344 rats and Syrian golden hamsters, keto-alcohol *14* and keto-aldehyde *9* are oxidized or reduced to the urinary metabolites *15*, *16* and *17* (Hecht *et al.*, 1980a; Hoffmann *et al.*, 1981). By pathways similar to those discussed above, α-hydroxylation of NNAl leads to the intermediates *5* and *6* and to the metabolites *16* and *17*. α-Hydroxylation of NNAl occurs more slowly than that of NNK (Castonguay *et al.*, 1983a).

The pyridine-*N*-oxide, NNK-1-*N*-oxide (*1*) is a microsomal metabolite of NNK and has been detected in the urine, serum and various tissues of Fischer 344 rats treated with NNK (Hecht *et al.*, 1980a; Castonguay *et al.*, 1983b). The pyridine *N*-oxide *2* has been detected in tissues of C57Bl mice treated with this compound (Castonguay *et al.*, 1984a).

Mutagenicity and other short-term tests

In the presence of a liver microsomal preparation from Aroclor-induced rats, NNK, at 1-4 μmol/plate, caused a dose-dependent increase in mutations in *Salmonella typhimurium* TA1535 and TA100 (Hecht *et al.*, 1983c).

NNK at 10^{-3}M and 10^{-2}M (1 and 10 mmol/ml) induced unscheduled DNA synthesis in freshly isolated hepatocytes from adult rats (Williams & Laspia, 1979).

O^6-Methylguanine and 7-methylguanine were detected in the DNA of liver and lung of Fischer 344 rats treated intravenously with 85 mg/kg bw NNK (Castonguay *et al.*, 1984b).

(*b*) *Humans*

No data were available to the Working Group on toxic effects or on effects on reproduction and prenatal toxicity.

Absorption, distribution, excretion and metabolism

Human tissues obtained at immediate autopsy and cultured for 24 h with [carbonyl-^{14}C]NNK metabolized NNK to NNAl and to compound *16* (see Fig. 1), as follows (values in nmol/100 ug DNA): buccal mucosa, 470 ± 273 and 0.26 ± 0.23; trachea, 315 ± 157 and 0.24 ± 0.13; oesophagus, 210 ± 163 and 0.14 ± 0.12; bronchus, 740 ± 581 and 0.86 ± 0.83; peripheral lung, 705 ± 398 and 0.37 ± 0.33; and urinary bladder, 398 ± 302 and 0.19 ± 0.28. Of the [carbonyl-^{14}C]NNK added to the medium, 50-80% was converted to NNAl. An unidentified metabolite was also formed (Castonguay *et al.*, 1983c).

No data were available to the Working Group on mutagenicity or chromosomal effects.

3.3 Case reports and epidemiological studies of carcinogenicity to humans

No data were available to the Working Group.

4. Summary of Data Reported and Evaluation

4.1 Exposure data

4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) has been found in a variety of tobacco products (chewing tobacco, snuff, cigarettes and cigars), in mainstream and sidestream smoke from cigars and cigarettes, in saliva of chewers of betel quid with tobacco, and in saliva of oral-snuff users. Some of the NNK in saliva appears to be formed endogenously from salivary nitrite and nicotine. Thus, there is widespread exposure to NNK among users of tobacco products and those exposed to sidestream smoke.

4.2 Experimental data

NNK was tested for carcinogenicity in several studies by subcutaneous injection in rats and hamsters and by intraperitoneal injection in mice. In rats, it induced carcinomas of the nasal cavity, lung and liver, with a clear dose-response relationship. In hamsters, it induced benign and malignant tumours of the nasal cavity, trachea and lung, even after a single administration. In mice, NNK and its metabolites 4-(methylnitrosamino)-1-(3-pyridyl-*N*-oxide)-1-butanone and 4-(methylnitrosamino)-1-(3-pyridyl)buan-1-ol induced benign and malignant tumours of the lung.

NNK and its metabolites can cross the placental barrier in mice. NNK can be metabolically activated by mouse foetal tissues.

Administration of NNK to rats results in abnormal DNA methylation in liver and lung. NNK is mutagenic to *Salmonella typhimurium* in the presence of an exogenous metabolic system. It induces unscheduled DNA synthesis in primary cultures of rat hepatocytes.

Overall assessment of data from short-term tests: 4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone[a]

	Genetic activity			Cell transformation
	DNA damage	Mutation	Chromosomal effects	
Prokaryotes		+		
Fungi/green plants				
Insects				
Mammalian cells (*in vitro*)	+			
Mammals (*in vivo*)	+			
Humans (*in vivo*)				
Degree of evidence in short-term tests for genetic activity: *Sufficient*				Cell transformation: No data

[a]The groups into which the table is divided and '+' are defined on pp. 16-17 of the Preamble; the degrees of evidence are defined on p. 18.

4.3 Human data

No case report or epidemiological study of the carcinogenicity of NNK to humans was available to the Working Group.

4.4 Evaluation[1]

There is *sufficient evidence*[2] for the carcinogenicity of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone to experimental animals.

No data on humans were available.

5. References

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[1]For description of the italicized term, see Preamble, pp. 15-16.

[2]In the absence of adequate data on humans, it is reasonable, for practical purposes, to regard chemicals for which there is *sufficient evidence* of carcinogenicity in animals as if they presented a carcinogenic risk to humans.

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